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Karyotypes of the Neotropical pseudoscorpions *Semeiochernes armiger* and *Cordylochernes scorpioides* (Pseudoscorpiones: Chernetidae)

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Abstract. The karyotypes and course of meiosis of two pseudoscorpions, *Semeiochernes armiger* (Balzan 1892) and *Cordylochernes scorpioides* (Linnaeus 1758) (Pseudoscorpiones: Chernetidae), are described for the first time. The diploid chromosome number of the male is 69 in *S. armiger* and 47 in *C. scorpioides*. As in most pseudoscorpions studied to date, autosomes exhibit predominantly biarmed morphology. Both species possess an X0 sex chromosome system. In most pseudoscorpions with X0 system karyotyped so far, including European chernetids, the X chromosome exhibits metacentric morphology. In contrast, the X chromosome of both neotropical chernetids studied exhibits asymmetric, submetacentric morphology.

Keywords: Pseudoscorpiones, Chernetidae, karyotype, sex chromosome, X0 sex chromosome determination

With more than 3,380 described species, Pseudoscorpiones is the fourth largest order of the arthropod class Arachnida (Harvey 2008). Despite this considerable species diversity, the morphology of pseudoscorpions is very conservative, and it can therefore be difficult to distinguish between closely related species on the basis of morphological features alone (Zeh & Zeh 1994; Wilcox et al. 1997; Zeh et al. 2003). Moreover, in many groups of pseudoscorpions, species identification is also complicated by the lack of detailed analysis of intraspecific morphological variability. For example, researchers originally described the neotropical pseudoscorpion, *Semeiochernes armiger* (Balzan 1892), from Central and South America as three species (*S. armiger*, *S. extraordinarius*, and *S. militaris*) on the basis of sexually dimorphic traits of the male pedipalpal chelae (Beier 1933, 1954). However, rearing experiments have demonstrated that intrapopulation variability encompasses the full range of *Semeiochernes* "interspecific" chelal morphology and that species status cannot be established using these male characters (Zeh & Zeh 1992).

Recent studies suggest that molecular and cytogenetic characters in pseudoscorpions diverge more rapidly than morphological traits and may thus prove particularly useful for identifying cryptic species and for resolving fine-scale evolutionary relationships. For example, the harlequin beetle riding pseudoscorpion, *Cordylochernes scorpioides* (Linnaeus 1758), ranging from Costa Rica to southern Brazil, was described by Beier (1948) as a single species, based on morphological examination of hundreds of specimens from several countries in South and Central America. However, mitochondrial cytochrome oxidase I (COI) gene sequencing has revealed extensive genetic differentiation, with a maximum likelihood nucleotide divergence of nearly 33% between *C. scorpioides* populations from Panama and northern South America (Trinidad and French Guiana) (Zeh et al. 2003). This extreme molecular divergence is associated with complete postzygotic incompatibility between individuals from central Panama and both French Guiana (Zeh & Zeh 1994) and Trinidad (J.A. Zeh, unpublished data), indicating that geographic populations of *C. scorpioides* constitute a complex of cryptic species. Interestingly, researchers have documented extensive mitochondrial COI sequence divergence between individuals from Panama and Trinidad in *S. armiger*, suggesting that the pattern exhibited by *C. scorpioides* may be common and that many neotropical pseudoscorpion species may actually represent cryptic species complexes (Wilcox et al. 1997).

Karyotype data also holds great promise as a tool for differentiating between closely related taxa. There is considerable karyotype diversity in all genera of pseudoscorpions that have been studied in detail to date, namely *Roncus* (Neobisiidae) (Troiano 1990, 1997), *Chthonius* (Chthoniidae) (Šťáhlavský & Král 2004), *Lasiochernes* (Chernetidae) (Šťáhlavský et al. 2005), *Geogarypus* (Geogarypidae), and *Olpium* (Olpiidae) (Šťáhlavský et al. 2006). In this paper, we present the results of the first cytogenetic study of *S. armiger* and *C. scorpioides*. Our karyotype analyses of these two neotropical representatives of the family Chernetidae not only contribute valuable data for comparing patterns of karyotype evolution in neotropical and European chernetid pseudoscorpions, but also provide a basis for future investigation of the relationship between karyotype evolution, molecular divergence, and speciation in *Semeiochernes* and *Cordylochernes*.

METHODS

All pseudoscorpions used in this study were derived from populations inhabiting decaying fig trees (*Ficus* spp.) in the lowland rain forest of the former Canal Zone, Republic of Panama (9°N, 79°W). Voucher specimens of *C. scorpioides* and *S. armiger* have been deposited with W.B. Muchmore (University of Rochester, USA), V. Mahnert (Muséum d'histoire naturelle, Geneva, Switzerland), and D. Quintero (Universidad de Panamá, Republic of Panama).

Semeiochernes armiger (Balzan 1892): 6 males collected in January 2006 either as adults (n = 3) or as tritonymphs that molted to the adult stage in the laboratory (n = 3).

Cordylochernes scorpioides (Linnaeus 1758): 8 males and 8 females from a large laboratory population established from 35 females collected in the field in August 2000.

Chromosome preparations were made using the technique described by Šťáhlavský & Král (2004). Briefly, gonads were dissected, hypotonised in 0.075 M KCl for 15 min, and fixed in a mixture of methanol:glacial acetic acid (3:1) for at least 20 min. We placed a piece of fixed material into a drop of 60% acetic acid on a clean microscope slide suspended by a pair of tungsten needles. Then we

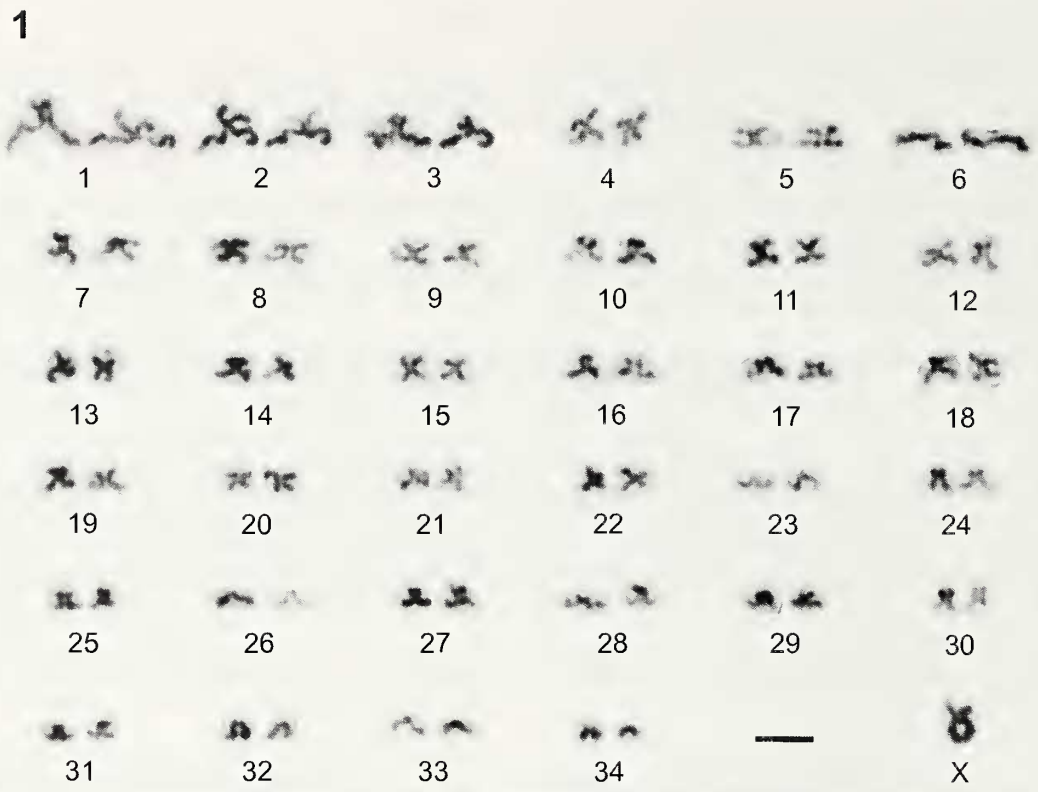


Figure 1.—*Semeiochernes armiger*, male karyotype. Based on two sister metaphase II plates. Bar = 5 μm.

transferred the slide onto a warm histological plate (surface temperature of 40–45° C) and moved the drop of dispersed tissue on the slide with a tungsten needle until it evaporated. The chromosome preparations were air-dried at room temperature overnight and stained with 5% Giemsa solution in Sörensen phosphate buffer (pH = 6.8) for 40 min.

Chromosome morphology was classified according to Levan et al. (1964). We calculated relative chromosome length as a percentage of the total length of the diploid set, including the sex chromosome. Owing to the small number of suitable spermatogonial mitotic metaphase plates, we used sister metaphase II for analysis of karyotypes in males. In addition, the centromere positions are much more obvious in pseudoscorpions at this meiotic stage.

RESULTS

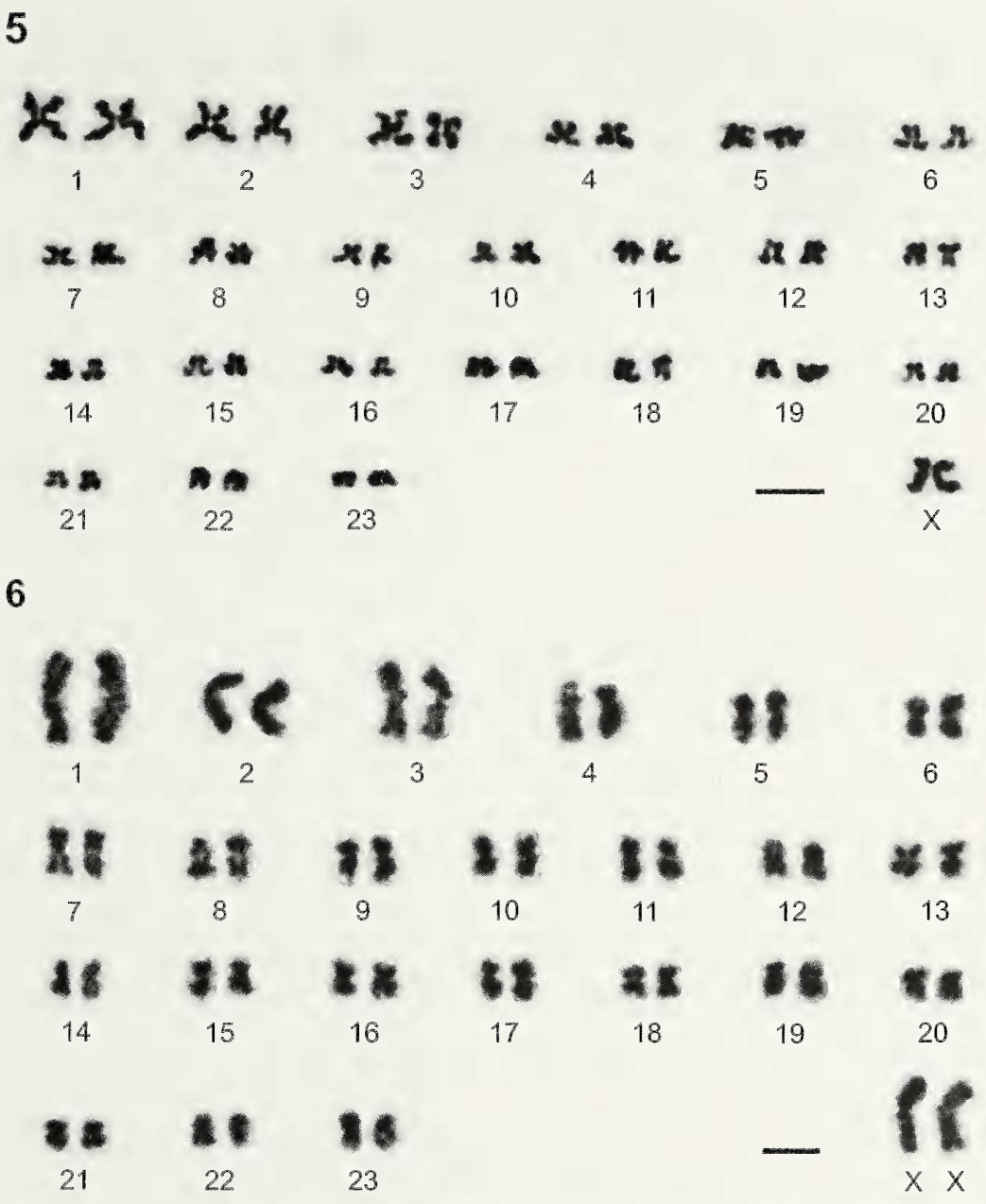
Semeiochernes armiger (Balzan 1892)

The male diploid complement comprises 69 chromosomes. The karyotype contains 18 pairs of metacentric (Nos. 4, 5, 8, 9, 11, 12, 14, 15, 16, 18, 19, 20, 21, 22, 25, 27, 30, 31), seven pairs of submetacentric (Nos. 1, 2, 7, 10, 13, 28, 29), three pairs of subtelocentric (Nos. 3, 17, 24), and six pairs of acrocentric (Nos. 6, 23, 26, 32, 33, 34) autosomes (Fig. 1). The first three pairs of autosomes are slightly larger than the other pairs (Fig. 1), and their relative size decreases from 3.4% to 2.5% of the diploid set. The remaining autosomes decrease gradually in size from 1.9% to 0.7% of the diploid set.

The sex chromosome system is X0. The X chromosome shows submetacentric morphology (centromeric index 1.83), constitutes 2.1% of the diploid set, and exhibits more intensive staining than other chromosomes (i.e., positive heteropycnosis) during some periods of meiotic division. During meiosis (Figs. 2–4), more intensive staining revealed overcondensation of the X chromosome from leptotene to pachytene (Fig. 2) and during metaphase II (Fig. 4). By contrast, we noted that all chromosomes are isopycnotic during metaphase I (Fig. 3).

Chiasma frequency is relatively low. In diplotene - metaphase I plates (n = 19), we observed at least one bivalent with two chiasmata and maximally four bivalents with two chiasmata. The mean chiasma frequency was 1.08 per bivalent.

Figures 2–4.—Course of meiosis in *Semeiochernes armiger*. 2. Pachytene. 3. Metaphase I. 4. Metaphase II cell containing X chromosome. Arrowheads indicate the X chromosome, arrow points to the bivalents with two chiasmata. Bars = 10 μm.



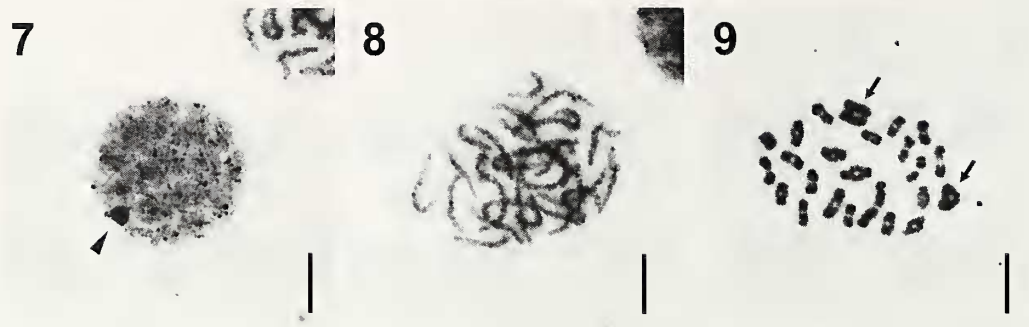
Figures 5–6.—*Cordylochernes scorpioides*. 5. Karyotype of male. Based on two sister metaphase II plates. 6. Karyotype of female. Based on mitotic metaphase. Bars = 5 μm.

Cordylochernes scorpioides (Linnaeus 1758)

The diploid number is 47 in males (Fig. 5) and 48 in females (Fig. 6). The karyogram of the species is based on two sister metaphases II of a male (Fig. 5). The karyotype is composed of 17 metacentric (Nos. 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, 16, 17, 18, 20, 21), three submetacentric (Nos. 8, 12, 15), and three subtelocentric (Nos. 19, 22, 23) pairs of autosomes (Fig. 5). The relative length of the autosomes decreases gradually from 4.4% to 1.4% of the diploid set in male metaphase II and from 4.8% to 1.2% of the diploid set in female mitotic metaphase. Comparison of the male and female karyotypes, as well as analysis of male meiosis, revealed an X0 sex chromosome system. The X chromosome is submetacentric (centromeric index 1.75) and relatively large, forming 3.6% of the diploid set in males and 2.7% in females. As in *S. armiger*, the X chromosome of *C. scorpioides* exhibits positive heteropycnosis in germinal cells of males. However, we detected heteropycnosis only during premeiotic interphase (Fig. 7). During late pachytene (Fig. 8), metaphase I (Fig. 9), and metaphase II (Fig. 5), the X chromosome appeared to be isopycnotic with the autosomes. During male meiosis, *C. scorpioides* exhibited lower chiasma frequency than *S. armiger* (number of analyzed diplotene plates = 378). We calculated the mean chiasma frequency as 1.03 per bivalent. More specifically, we observed two bivalents with two chiasmata in five diplotene nuclei and one bivalent with three chiasmata in five diplotene nuclei. In the remaining cells, all bivalents had only one chiasma.

DISCUSSION

Until now, karyotype descriptions of the family Chernetidae have been limited to five species, all from the European region. Sokolow (1926) published basic data on the karyotype of *Dendrochernes cyrneus* (L. Koch 1873) in his pioneering analysis of spermatogenesis in pseudoscorpions. However, nearly 80 years elapsed before



Figures 7–9.—Course of meiosis in *Cordylochernes scorpioides*. 7. Premeiotic interphase. 8. Late pachytene. 9. Metaphase I. Arrowhead indicates the X chromosome, arrows point to the bivalents with two chiasmata. Bars = 10 μm.

scientists conducted any further karyotype analyses of chernetid species. In a study aimed at reconstructing the phylogeny of arthropod telomeric sequences, Vítková et al. (2005) provided only data on the diploid number and telomeric sequences of *Chernes hahnii* (C.L. Koch 1839). Šťáhlavský et al. (2005) provided the first detailed descriptions of chernetid karyotypes, which were limited to three species in the genus *Lasiochernes*. Despite limited comparative data, European chernetids appear to be characterized by high diploid chromosome number (49–73), a predominance of biarmed chromosomes, and the presence of an X0 sex chromosome system (Šťáhlavský et al. 2005).

In this study, we present the first karyotype descriptions of pseudoscorpions from the Neotropics. As with previously karyotyped pseudoscorpions (e.g., Troiano 1997; Šťáhlavský & Král 2004; Šťáhlavský et al. 2005, 2006), neotropical chernetids exhibit considerable karyotype variability. The diploid chromosome number of the *C. scorpioides* male (2n = 47) is the lowest known diploid number within chernetids. By contrast, the male karyotype of *S. armiger* consists of 69 chromosomes. Despite this disparity in chromosome number, the karyotypes of both neotropical species have several features in common with European chernetids. Their karyotypes are characterized by high 2n, as well as by a predominance of biarmed chromosomes. As with the majority of pseudoscorpions karyotyped so far, all representatives of the family Chernetidae possess an X0 sex chromosome system. Remarkably, European and neotropical chernetids differ in the morphology and relative size of the sex chromosome. In all karyotyped European species, the sex chromosome is metacentric and is the largest element of the karyotype (Šťáhlavský et al. 2005). Metacentric morphology of the X chromosome has been found in the majority of pseudoscorpions exhibiting the X0 sex chromosome system (Troiano 1990, 1997; Šťáhlavský & Král 2004; Šťáhlavský et al. 2005, 2006). Interestingly, in both neotropical chernetids, the X chromosome exhibits submetacentric morphology. Moreover, it is not the largest element of the karyotype. These fundamental differences may be the result of long-term isolation and divergent evolution of the X chromosome in European and neotropical chernetids. Clearly, many additional cytogenetic and molecular systematic studies of species and populations from both biogeographical regions are needed in order to gain a better understanding of karyotype evolution and diversity in chernetid pseudoscorpions.

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